Using asreml and asremlPlus for the Ladybird example from Welham et al. (2014)

Chris Brien

19 March, 2021

Introduction

This vignette shows how to use asremlPlus (Brien, 2021a), and dae (Brien, 2021b), for exploring and presenting predictions from a linear mixed model analysis, the predictions having been produced using asreml (Butler et al., 2020). Here, asremlPlus, dae and asreml are packages for the R Statistical Computing environment (R Core Team, 2021).

The context is a three-factor factorial experiment on ladybirds (Welham et al., 2014, Example 8.2) that aims to answer the question "Will ladybirds transfer fungus to aphids on plants?" The experiment consists of 2 runs of 36 containers, each with a plant and aphids. There are three factors that results in 12 treatments: Host plant (beans, trefoil), infected Cadavers (5, 10, 20), Ladybird (-, +). Ther are randomized to the containers within a run so that each is replicated 3 times within a run. The respose to be analysed is the logit of the proportion of live aphids that were infected.

Initialize

```
library(knitr)
opts_chunk$set("tidy" = FALSE, comment = NA)
suppressMessages(library(asreml))
packageVersion("asreml")

## [1] '4.1.0.130'
suppressMessages(library(asremlPlus))
packageVersion("asremlPlus")

## [1] '4.2.32'
suppressMessages(library("dae"))
packageVersion("dae")

## [1] '3.1.37'
options(width = 95, show.signif.stars = FALSE)
```

Get data available in asremlPlus

```
data("Ladybird.dat")
```

Do an ANOVA of logits

```
Ladybird.aov <- aov(logitP ~ Host*Cadavers*Ladybird + Error(Run/Plant),</pre>
                    data=Ladybird.dat)
summary(Ladybird.aov)
Error: Run
          Df Sum Sq Mean Sq F value Pr(>F)
Residuals 1 0.06766 0.06766
Error: Run:Plant
                       Df Sum Sq Mean Sq F value
                        1 13.599 13.599 59.172 1.82e-10
Host
Cadavers
                        2 17.027
                                  8.514 37.044 3.78e-11
Ladybird
                        1 11.091 11.091 48.257 3.33e-09
Host:Cadavers
                        2 0.308
                                  0.154
                                           0.670
                                                   0.5158
Host:Ladybird
                        1 0.228
                                   0.228
                                           0.992
                                                   0.3234
Cadavers:Ladybird
                        2 1.735
                                   0.867
                                           3.774
                                                   0.0287
Host:Cadavers:Ladybird 2 0.200
                                   0.100
                                           0.435
                                                   0.6493
Residuals
                       59 13.560
                                   0.230
```

The anova table gives the F-tests for the three-factor effects and interactions. Note the Residuals Mean Sq value for Run:Plant of 0.230. Also, it is clear that the Run component is negative, given that the Residuals Mean Sq value for Run is less than that for Run:Plant; it is (0.06766 - 0.230) / 36). From the table it is seen that the only significant interaction is Cadavers:Ladybird and that the Host main effect is significant.

Use asreml to analyse the logits

Mixed model analysis of logits

License check Fri Mar 19 11:36:30 2021

Model fitted using the gamma parameterization.

ASReml 4.1.0 Fri Mar 19 11:36:32 2021

	${ t LogLik}$	Sigma2	DF wall	cpu
1	3.07130	0.226239	60 11:36:32	0.0 (1 restrained)
2	3.64260	0.226912	60 11:36:32	0.0 (1 restrained)
3	3.71250	0.227106	60 11:36:32	0.0 (1 restrained)
4	3.71721	0.227121	60 11:36:32	0.0 (1 restrained)
5	3.71751	0.227122	60 11:36:32	0.0 (1 restrained)

Warning in asreml(logitP \sim Host * Cadavers * Ladybird, random = \sim Run, residual = \sim Run:Plant, : Some components changed by more than 1% on the last iteration.

```
summary(m)$varcomp
```

```
component std.error z.ratio bound %ch
Run 2.298309e-08 NA NA B 93.7
Run:Plant!R 2.271216e-01 0.04156985 5.463612 P 0.0
```

As expected the Run component is bound (B) at approximately zero. This results in a change in the estimate of the residual variance to 0.227. To allow for a negative estimate we will unconstrain the Run component.

As Littell et al. (2006, p.150) say

if you do not set the negative variance component estimate to zero, but allow it to remain negative, you get better control over Type I error and, for cases of negative wholeplot error variance estimates, greater power. Therefore, this is the recommended procedure.

Unconstrain Reps to make the analysis equivalent to ANOVA

```
m <- setvarianceterms(m$call, terms = "Run", bounds = "U")</pre>
```

Model fitted using the gamma parameterization.

ASReml 4.1.0 Fri Mar 19 11:36:32 2021

	LogLik	Sigma2	DF wall	cpu
1	3.07130	0.226239	60 11:36:32	0.0 (1 restrained)
2	3.64260	0.226912	60 11:36:32	0.0 (1 restrained)
3	3.80283	0.227454	60 11:36:32	0.0 (1 restrained)
4	3.83949	0.233418	60 11:36:32	0.0
5	3.95568	0.230971	60 11:36:32	0.0
6	3.97333	0.230035	60 11:36:32	0.0
7	3.97405	0.229835	60 11:36:32	0.0

summary(m)\$varcomp

```
component std.error z.ratio bound %ch
Run -0.004504789 0.002896281 -1.555370 U 0.1
Run:Plant!R 0.229834648 0.042316936 5.431269 P 0.0
```

Now the Run component is negative and the Run:Plant variance estimate is now equal to that for the Residuals Mean Sq for Run:Plant from the anova table.

Set up an asrtests object

```
current.asrt <- as.asrtests(m)

Calculating denominator DF
print(current.asrt, which = "pseudoanova")</pre>
```

Pseudo-anova table for fixed terms

Wald tests for fixed effects.

Response: logitP

	Df	denDF	F.inc	Pr
(Intercept)	1	1	1550.00	0.0162
Host	1	59	59.17	0.0000
Cadavers	2	59	37.04	0.0000
Ladybird	1	59	48.26	0.0000
Host:Cadavers	2	59	0.67	0.5158
Host:Ladybird	1	59	0.99	0.3234
Cadavers:Ladybird	2	59	3.77	0.0287
${\tt Host:Cadavers:Ladybird}$	2	59	0.44	0.6493

The asrtests object contains a wald.tab component which can be printed by specifying that the pseudoanova is printed. The F-values for the fixed terms in this table are the same as those in the anova table.

Obtain the marginality matrix for the fixed terms

The pstructure function from the dae package (Brien, 2021) produce the marginality matrix for a formula as a side effect and we take advantage of that to obtain the matrix required here.

	Host	Cadavers	Host:Cadavers	Ladybird	Host:Ladybird	Cadavers:Ladybird
Host	1	0	1	0	1	0
Cadavers	0	1	1	0	0	1
Host:Cadavers	0	0	1	0	0	0
Ladybird	0	0	0	1	1	1
Host:Ladybird	0	0	0	0	1	0
Cadavers:Ladybird	0	0	0	0	0	1
Host:Cadavers:Ladybird	. 0	0	0	0	0	0

Host:Cadavers:Ladybird

Host	1
Cadavers	1
Host:Cadavers	1
Ladybird	1
Host:Ladybird	1
Cadavers:Ladybird	1
Host:Cadavers:Ladybird	1

This marginality matrix is interpreted by taking a row term and noting that it is marginal to any column term with a one in this row.

Choose marginality-compliant model

```
chosen <- chooseModel(current.asrt, terms.marginality = HCL.marg)

Calculating denominator DF
current.asrt <- chosen$asrtests.obj
print(current.asrt, which = "test", omit.columns = c("AIC", "BIC"))</pre>
```

Sequence of model investigations

```
terms DF denDF p action

1 Host:Cadavers:Ladybird 2 59 0.6493 Nonsignificant

2 Cadavers:Ladybird 2 59 0.0287 Significant

3 Host:Ladybird 1 59 0.3234 Nonsignificant

4 Host:Cadavers 2 59 0.5158 Nonsignificant

5 Host 1 59 0.0000 Significant

(chosen$sig.terms)
```

[[1]]

[1] "Cadavers:Ladybird"

```
[[2]]
[1] "Host"
```

The chooseModel function produces a list with components sig.terms, a list with the terms in the marginality-compliant model, and asrtests.obj, the asrtests object resulting from the model selection. In particular, the asrtests object contains a test.summary that details the tests performed in choosing the model; the AIC and BIC columns are omitted from test.summary because their inclusion has not been requested. Note that chooseModel does not test the main effects for Cadavers or Ladybird, because these are marginal to the significant two-factor interaction Cadavers:Ladybird.

Form formula for selected model

```
chosen.mod <- paste(unlist(chosen$sig.terms), collapse = " + ")
(chosen.mod <- as.formula(paste("~", chosen.mod)))</pre>
```

~Cadavers:Ladybird + Host

Obtain predictions under the chosen model and form an alldiffs object

Predictions for logitP transform(s) from Host:Ladybird:Cadavers

Notes:

The original predictions, obtained as described below, have been linearly transformed to form estimated marginal means.

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

	Host	${\tt Ladybird}$	${\tt Cadavers}$	<pre>predicted.value</pre>	${\tt standard.error}$	upper.halfLeastSignificant.limit
1	bean	_	5	-1.6038338	0.1417454	-1.4080222
2	bean	_	10	-1.1454308	0.1417454	-0.9496192
3	bean	-	20	-0.7448097	0.1417454	-0.5489981
4	bean	+	5	-1.0195475	0.1417454	-0.8237359
5	bean	+	10	-0.5983440	0.1417454	-0.4025323
6	bean	+	20	0.4786704	0.1417454	0.6744820
7	trefoil	-	5	-2.4730339	0.1417454	-2.2772223
8	trefoil	-	10	-2.0146309	0.1417454	-1.8188193
9	trefoil	-	20	-1.6140098	0.1417454	-1.4181982
10	trefoil	+	5	-1.8887476	0.1417454	-1.6929360
11	trefoil	+	10	-1.4675441	0.1417454	-1.2717325
12	trefoil	+	20	-0.3905297	0.1417454	-0.1947181

lower.halfLeastSignificant.limit est.status

```
1
                         -1.7996454 Estimable
2
                         -1.3412425 Estimable
3
                         -0.9406214 Estimable
4
                         -1.2153592 Estimable
5
                         -0.7941556 Estimable
6
                         0.2828588 Estimable
7
                         -2.6688455 Estimable
                         -2.2104426 Estimable
8
9
                         -1.8098215 Estimable
10
                         -2.0845593 Estimable
11
                         -1.6633557 Estimable
12
                         -0.5863414 Estimable
```

LSD values

```
minimum LSD = 0.3916233 0.3916233

mean LSD = 0.3916233 0.3916233

maximum LSD = 0.3916233 0.3916233

(sed range / mean sed = 5.67e-16 7.09e-16)
```

Setting the terms argument to Host:Ladybird:Cadavers requests predictions for all combinations of the three factors and the linear.transformation argument is used to obtain estimated marginal means (emm) that conform to the chosen model. The wald.tab is supplied so that it can be used to get the degrees of freedom for the t-value to be used in calculating the LSD; the degrees of freedom ot the source for the terms argument will be used. The error.intervals argument has been set to "halfLeast", the meanLSD.type argument to "factor.combination" and the LSDby argument to "Host" so that the average LSD will be calculated for each Host. This necessary because, under the chosen model, the LSDs differ between Hosts. It results in lower.halfLeastSignificant.limit and upper.halfLeastSignificant.limit being added to the predictions component of the alldiffs object.

Or, caclulate predictions to check first and then transform to conform to chosen model

Predictions for logitP transform(s) from Host:Ladybird:Cadavers

Notes:

The original predictions, obtained as described below, have been linearly transformed to form estimated marginal means.

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.
- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

```
Host Ladybird Cadavers predicted.value standard.error upper.halfLeastSignificant.limit
1
     bean
                          5
                                 -1.6038338
                                                 0.1417454
                                                                                 -1.4080222
2
     bean
                         10
                                 -1.1454308
                                                 0.1417454
                                                                                 -0.9496192
3
     bean
                         20
                                 -0.7448097
                                                 0.1417454
                                                                                 -0.5489981
4
     bean
                         5
                                 -1.0195475
                                                 0.1417454
                                                                                 -0.8237359
5
                        10
     bean
                                 -0.5983440
                                                 0.1417454
                                                                                 -0.4025323
                        20
6
     bean
                                 0.4786704
                                                 0.1417454
                                                                                  0.6744820
7 trefoil
                                                                                 -2.2772223
                        5
                                 -2.4730339
                                                 0.1417454
                        10
8 trefoil
                                 -2.0146309
                                                 0.1417454
                                                                                 -1.8188193
9 trefoil
                         20
                                 -1.6140098
                                                 0.1417454
                                                                                 -1.4181982
10 trefoil
                 +
                         5
                                 -1.8887476
                                                 0.1417454
                                                                                 -1.6929360
11 trefoil
                         10
                                 -1.4675441
                                                 0.1417454
                                                                                 -1.2717325
12 trefoil
                         20
                                 -0.3905297
                                                 0.1417454
                                                                                 -0.1947181
  lower.halfLeastSignificant.limit est.status
1
                        -1.7996454 Estimable
2
                        -1.3412425 Estimable
3
                        -0.9406214 Estimable
```

```
4
                        -1.2153592 Estimable
                        -0.7941556 Estimable
5
6
                        0.2828588 Estimable
7
                        -2.6688455 Estimable
8
                        -2.2104426 Estimable
9
                        -1.8098215 Estimable
                        -2.0845593 Estimable
10
                        -1.6633557 Estimable
11
12
                        -0.5863414 Estimable
```

LSD values

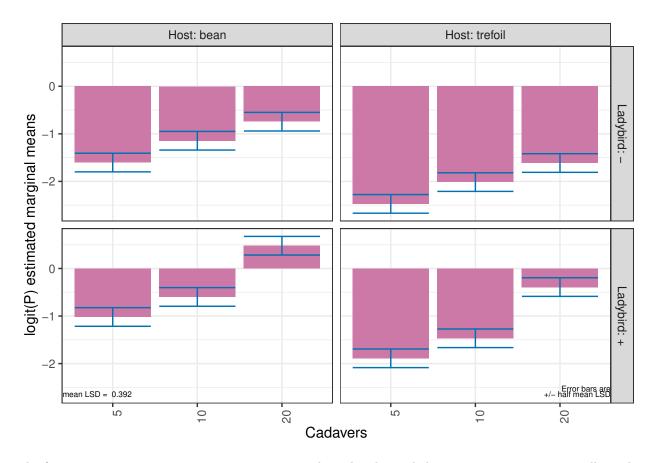
```
minimum LSD = 0.3916233 0.3916233

mean LSD = 0.3916233 0.3916233

maximum LSD = 0.3916233 0.3916233

(sed range / mean sed = 5.67e-16 7.09e-16)
```

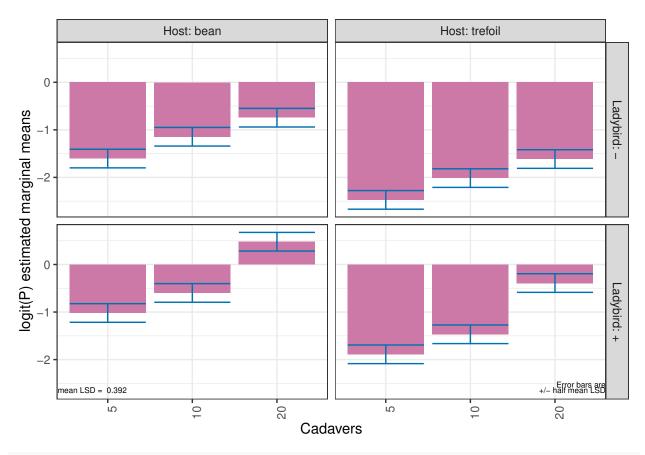
Plot the predictions



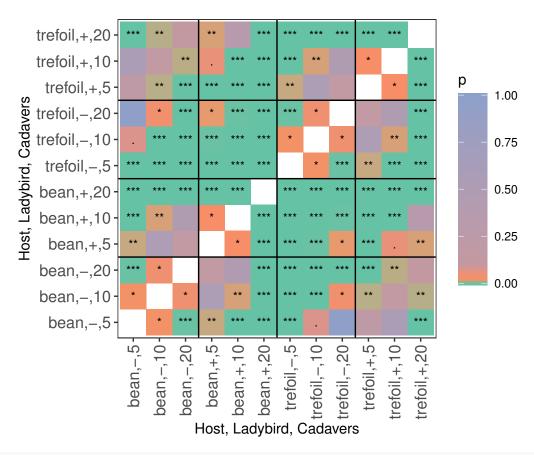
The function plotPredictions uses ggplot to produce the plot and the ggplotFuncs argument allows the addition of ggplot functions to modify the plot. In this case, the facet.grid function is respecified to include prepender functions that modify the labels of the facets to include the factor names. Note the the error bars in the plots are of $\pm 0.5 LSD$ so that pairs of prediction with nonoverlapping bars are significantly different (Snee, 1981).

Get and plot the predictions with a single function call

The predictPresent function combines the functionality of predictPlus and plotPredictions, as demonstrated now. Also, the use of plotPvalues to plot the pairwise p-values is displayed. The predictPresent function has the capability of producing alldiffs objects for multiple terms and these are stored in a list each of which is named for the term whose alldiffs object it stores. Thus, the term has to be specified in referencing components of diffs.



plotPvalues(diffs\$Host.Ladybird.Cadavers, factors.per.grid = 1, show.sig = TRUE)



options(width = 90) diffs\$Host.Ladybird.Cadavers\$differences

```
bean,-,5 bean,-,10 bean,-,20
                                                   bean,+,5 bean,+,10 bean,+,20
bean, -, 5
              0.00000000 - 0.4584030 - 0.8590241 - 0.5842863 - 1.0054898 - 2.0825042
bean, -, 10
              0.45840297
                           0.0000000 - 0.4006211 - 0.1258833 - 0.5470869 - 1.6241012
bean, -, 20
                                                  0.2747378 -0.1464657 -1.2234801
              0.85902408
                           0.4006211
                                     0.0000000
bean,+,5
              0.58428627
                           0.1258833 -0.2747378
                                                 0.0000000 -0.4212036 -1.4982179
                                                            0.0000000 -1.0770144
bean, +, 10
              1.00548982
                           0.5470869
                                      0.1464657
                                                  0.4212036
                                                             1.0770144
bean, +, 20
              2.08250420
                           1.6241012
                                      1.2234801
                                                  1.4982179
                                                                        0.0000000
trefoil,-,5
             -0.86920012 -1.3276031 -1.7282242 -1.4534864 -1.8746899 -2.9517043
trefoil,-,10 -0.41079715 -0.8692001 -1.2698212 -0.9950834 -1.4162870 -2.4933014
trefoil,-,20 -0.01017604 -0.4685790 -0.8692001 -0.5944623 -1.0156659 -2.0926802
             -0.28491385 -0.7433168 -1.1439379 -0.8692001 -1.2904037 -2.3674180
trefoil,+,5
trefoil,+,10
              0.13628970 -0.3221133 -0.7227344 -0.4479966 -0.8692001 -1.9462145
trefoil,+,20
              1.21330408
                          0.7549011 0.3542800 0.6290178 0.2078143 -0.8692001
             trefoil,-,5 trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -, 5
               0.8692001
                             0.4107972
                                         0.01017604
                                                       0.2849139
                                                                    -0.1362897
                                                                                 -1.2133041
               1.3276031
                             0.8692001
                                         0.46857901
                                                       0.7433168
                                                                     0.3221133
                                                                                 -0.7549011
bean, -, 10
                                                                                 -0.3542800
                                                                     0.7227344
bean, -, 20
               1.7282242
                             1.2698212
                                         0.86920012
                                                       1.1439379
                                                                                 -0.6290178
bean,+,5
               1.4534864
                             0.9950834
                                          0.59446231
                                                       0.8692001
                                                                     0.4479966
bean, +, 10
               1.8746899
                             1.4162870
                                          1.01566586
                                                       1.2904037
                                                                     0.8692001
                                                                                 -0.2078143
bean, +, 20
               2.9517043
                             2.4933014
                                          2.09268024
                                                       2.3674180
                                                                     1.9462145
                                                                                  0.8692001
trefoil,-,5
               0.000000
                            -0.4584030
                                        -0.85902408
                                                      -0.5842863
                                                                    -1.0054898
                                                                                 -2.0825042
trefoil,-,10
               0.4584030
                             0.000000
                                        -0.40062111
                                                      -0.1258833
                                                                    -0.5470869
                                                                                 -1.6241012
trefoil,-,20
                                         0.00000000
                                                       0.2747378
               0.8590241
                             0.4006211
                                                                    -0.1464657
                                                                                 -1.2234801
```

```
trefoil,+,5
              0.5842863
                           0.1258833 -0.27473781
                                                    0.0000000
                                                                 -0.4212036
                                                                              -1.4982179
               1.0054898
                                                                  0.0000000
trefoil,+,10
                           0.5470869
                                        0.14646574
                                                    0.4212036
                                                                              -1.0770144
trefoil,+,20
                                                     1.4982179
                                                                  1.0770144
              2.0825042
                            1.6241012
                                        1.22348012
                                                                               0.0000000
options(width = 90)
print(diffs$Host.Ladybird.Cadavers$sed)
             bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
                   NA 0.1957142 0.1957142 0.1957142 0.1957142 0.1957142
bean, -, 5
                                                                          0.1129957
bean,-,10
                             NA 0.1957142 0.1957142 0.1957142 0.1957142
                                                                          0.2259913
            0.1957142
bean, -, 20
            0.1957142 0.1957142
                                       NA 0.1957142 0.1957142 0.1957142
                                                                          0.2259913
bean,+,5
            0.1957142 0.1957142 0.1957142
                                                 NA 0.1957142 0.1957142
                                                                          0.2259913
bean,+,10
            0.1957142 0.1957142 0.1957142 0.1957142
                                                                          0.2259913
            0.1957142\ 0.1957142\ 0.1957142\ 0.1957142\ 0.1957142
bean,+,20
                                                                          0.2259913
trefoil,-,5 0.1129957 0.2259913 0.2259913 0.2259913 0.2259913 0.2259913
                                                                                 NA
trefoil,-,10 0.2259913 0.1129957 0.2259913 0.2259913 0.2259913 0.2259913
                                                                          0.1957142
trefoil,-,20 0.2259913 0.2259913 0.1129957 0.2259913 0.2259913 0.2259913
                                                                          0.1957142
trefoil,+,5 0.2259913 0.2259913 0.1129957 0.2259913 0.2259913
                                                                          0.1957142
trefoil,+,10 0.2259913 0.2259913 0.2259913 0.1129957 0.2259913
                                                                          0.1957142
trefoil,+,20 0.2259913 0.2259913 0.2259913 0.2259913 0.2259913 0.1129957
                                                                          0.1957142
            trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -, 5
                0.2259913
                             0.2259913
                                        0.2259913
                                                     0.2259913
                                                                   0.2259913
bean,-,10
                0.1129957
                            0.2259913
                                        0.2259913
                                                     0.2259913
                                                                   0.2259913
bean,-,20
                            0.1129957
                                                     0.2259913
                                                                   0.2259913
               0.2259913
                                        0.2259913
bean,+,5
               0.2259913
                            0.2259913
                                        0.1129957
                                                     0.2259913
                                                                   0.2259913
bean,+,10
               0.2259913
                            0.2259913
                                        0.2259913
                                                     0.1129957
                                                                   0.2259913
bean, +, 20
               0.2259913
                            0.2259913
                                       0.2259913
                                                     0.2259913
                                                                   0.1129957
trefoil,-,5
               0.1957142
                            0.1957142
                                        0.1957142
                                                     0.1957142
                                                                   0.1957142
                            0.1957142
trefoil,-,10
                      NA
                                        0.1957142
                                                     0.1957142
                                                                   0.1957142
trefoil,-,20
               0.1957142
                                        0.1957142
                                                     0.1957142
                                                                   0.1957142
                                   NA
               0.1957142
                            0.1957142
                                                     0.1957142
trefoil,+,5
                                               NA
                                                                   0.1957142
trefoil,+,10
                0.1957142
                             0.1957142
                                        0.1957142
                                                            NA
                                                                   0.1957142
                            0.1957142
trefoil,+,20
               0.1957142
                                        0.1957142
                                                     0.1957142
                                                                          NA
```

Perform the analysis with just selected model fitted

The model with nonsignificant fixed terms dropped is obtained in order to compare it with the fit when they are retained and the etimated marginal means for the chosen model are obtained.

Pseudo-anova table for fixed terms

Wald tests for fixed effects.

Response: logitP

```
Df denDF F.inc
(Intercept)
                  1
                       1 1550.00 0.0162
Host
                  1
                       64 60.88 0.0000
Cadavers
                  2
                       64 38.12 0.0000
Ladybird
                       64 49.65 0.0000
                  1
Cadavers:Ladybird 2
                       64
                            3.88 0.0256
diffs.red <- predictPlus(red.asrt$asreml.obj,</pre>
                        classify = "Host:Ladybird:Cadavers",
                        wald.tab = current.asrt$wald.tab,
                        error.intervals = "halfLeast",
                        meanLSD.type = "factor.combination", LSDby = "Host",
```

Predictions for logitP from Host:Ladybird:Cadavers

Notes:

- The predictions are obtained by averaging across the hypertable calculated from model terms constructed solely from factors in the averaging and classify sets.

tables = "predictions")

- Use 'average' to move ignored factors into the averaging set.
- The ignored set: Run

	Host	Ladybird	${\tt Cadavers}$	predicted.value	standard.error
1	bean	-	5	-1.6038338	0.1398332
2	bean	-	10	-1.1454308	0.1398332
3	bean	-	20	-0.7448097	0.1398332
4	bean	+	5	-1.0195475	0.1398332
5	bean	+	10	-0.5983440	0.1398332
6	bean	+	20	0.4786704	0.1398332
7	trefoil	-	5	-2.4730339	0.1398332
8	trefoil	-	10	-2.0146309	0.1398332
9	trefoil	-	20	-1.6140098	0.1398332
10	trefoil	+	5	-1.8887476	0.1398332
11	${\tt trefoil}$	+	10	-1.4675441	0.1398332
12	${\tt trefoil}$	+	20	-0.3905297	0.1398332
	upper.ha	alfLeastS:	ignificant	t.limit lower.ha	lfLeastSignifica
1			-1.4	4107942	-1
2			0 (NEO2012	4

ant.limit est.status 1.7968734 Estimable -1.3384704 Estimable -0.9523913 -0.9378493 Estimable 3 -0.5517702 4 -0.8265080 -1.2125871 Estimable -0.7913835 Estimable 5 -0.4053044 6 0.6717100 0.2856308 Estimable 7 -2.6660735 Estimable -2.2799944 -1.8215914 8 -2.2076705 Estimable 9 -1.8070494 Estimable -1.4209703-1.6957081 10 -2.0817872 Estimable 11 -1.2745045 -1.6605837 Estimable 12 -0.1974902 -0.5835693 Estimable

```
LSD values
minimum LSD = 0.3860791 0.3860791
            0.3860791 0.3860791
mean LSD =
maximum LSD = 0.3860791 0.3860791
(sed range / mean sed = 2.44e-15 2.3e-15)
options(width = 90)
print(diffs.red$sed)
              bean,-,5 bean,-,10 bean,-,20 bean,+,5 bean,+,10 bean,+,20 trefoil,-,5
                    NA 0.1929435 0.1929435 0.1929435 0.1929435
bean, -, 5
                                                                             0.1113960
bean, -, 10
             0.1929435
                              NA 0.1929435 0.1929435 0.1929435 0.1929435
                                                                             0.2227920
bean, -, 20
             0.1929435 0.1929435
                                         NA 0.1929435 0.1929435 0.1929435
                                                                             0.2227920
bean,+,5
             0.1929435 0.1929435 0.1929435
                                                   NA 0.1929435 0.1929435
                                                                             0.2227920
             0.1929435 0.1929435 0.1929435 0.1929435
                                                              NA 0.1929435
                                                                             0.2227920
bean,+,10
bean,+,20
             0.1929435 0.1929435 0.1929435 0.1929435 0.1929435
                                                                             0.2227920
                                                                        NA
trefoil,-,5
             0.1113960 0.2227920 0.2227920 0.2227920 0.2227920 0.2227920
                                                                                    NA
trefoil,-,10 0.2227920 0.1113960 0.2227920 0.2227920 0.2227920 0.2227920
                                                                             0.1929435
trefoil,-,20 0.2227920 0.2227920 0.1113960 0.2227920 0.2227920 0.2227920
                                                                             0.1929435
trefoil,+,5 0.2227920 0.2227920 0.1113960 0.2227920 0.2227920
                                                                             0.1929435
trefoil,+,10 0.2227920 0.2227920 0.2227920 0.2227920 0.1113960 0.2227920
                                                                             0.1929435
trefoil,+,20 0.2227920 0.2227920 0.2227920 0.2227920 0.2227920 0.1113960
                                                                             0.1929435
             trefoil,-,10 trefoil,-,20 trefoil,+,5 trefoil,+,10 trefoil,+,20
bean, -, 5
                0.2227920
                              0.2227920
                                          0.2227920
                                                       0.2227920
                                                                     0.2227920
                0.1113960
                             0.2227920
                                          0.2227920
                                                       0.2227920
                                                                     0.2227920
bean, -, 10
                             0.1113960
bean, -, 20
                0.2227920
                                          0.2227920
                                                       0.2227920
                                                                     0.2227920
                0.2227920
                              0.2227920
                                          0.1113960
                                                       0.2227920
                                                                     0.2227920
bean, +, 5
bean, +, 10
                0.2227920
                              0.2227920
                                          0.2227920
                                                       0.1113960
                                                                     0.2227920
                0.2227920
                             0.2227920
                                                       0.2227920
bean, +, 20
                                          0.2227920
                                                                     0.1113960
trefoil,-,5
                0.1929435
                             0.1929435
                                          0.1929435
                                                       0.1929435
                                                                     0.1929435
trefoil,-,10
                       NA
                              0.1929435
                                          0.1929435
                                                       0.1929435
                                                                     0.1929435
trefoil,-,20
                0.1929435
                                          0.1929435
                                                       0.1929435
                                     NA
                                                                     0.1929435
trefoil,+,5
                0.1929435
                             0.1929435
                                                 NA
                                                       0.1929435
                                                                     0.1929435
trefoil,+,10
                0.1929435
                              0.1929435
                                          0.1929435
                                                               NA
                                                                     0.1929435
```

References

0.1929435

0.1929435

trefoil,+,20

Brien, C. J. (2021a) asremlPlus: Augments ASReml-R in fitting mixed models and packages generally in exploring prediction differences. Version 4.2-32. https://cran.r-project.org/package=asremlPlus/ or http://chris.brien.name/rpackages/.

0.1929435

0.1929435

NA

Brien, C. J. (2021b) dae: Functions useful in the design and ANOVA of experiments. Version 3.1-37. https://cran.r-project.org/package=dae/ or http://chris.brien.name/rpackages/.

Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J. and Thompson, R. (2020). ASReml-R Reference Manual Version 4.1.0.130. VSN International Ltd, https://asreml.kb.vsni.co.uk/.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., & Schabenberger, O. (2006). SAS for Mixed Model. (2nd ed.). Cary, N.C.: SAS Press.

R Core Team (2021) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/.

Snee, R. D. (1981). Graphical Display and Assessment of Means. Biometrics, 37, 835–836.

Welham, S. J., Gezan, S. A., Clark, S. J., & Mead, A. (2014). Statistical Methods in Biology: Design and Analysis of Experiments and Regression. Boca Raton: Chapman and Hall/CRC.